# SENSING GASEOUS SUBSTANCES USING METAL COMPLEXES

The present invention concerns improvements in sensors, and more particularly concerns improvements in sensors for detecting microbial food spoilage.

Microbial spoilage of foods is a major concern to food producers, retailers and consumers. Consumers may perceive spoilage as a deterioration in taste, appearance, smell and/or texture, and there are clear health risks too. Currently, there is no direct in-pack measurement of food spoilage. Producers/retailers use "best before" and "use by" dates as an indication of food quality and safety. However, these methods are merely a prediction of food quality and are not a real measurement of food quality.

Food can spoil by a number of processes, including lipid oxidation, enzymatic degradation and microbial growth. The relative importance of these food spoilage processes vary from food to food, according to its constitution, handling history, and other factors. Microbial growth, however, is a major spoilage factor.

There are many methods currently used to determine food quality, *eg* organoleptic tests, standard microbiological techniques and spectroscopic analysis. None of these techniques are currently suitable for use in-pack, and may have other disadvantages such as long evaluation times and sample destruction. Accordingly, there is a need for a technique which can continuously monitor food quality in-pack, from packaging to consumption.

It has been proposed to use a fluorophore chelated with manganese for the quantitative detection of S-containing pesticides (Int. J. Environ. Chem. (1971), 1 (2), 99-111). Also, the fluorophore calcein has been described as being complexed with palladium with added zinc, to detect organo-sulphur drug residue compounds in chromatography techniques (J. Chromat. 442 (1988) 459-463) in which the compounds are spotted onto thin layer chromatography plates.

It has also been suggested that the concentration of sulphur-containing vapours from dry-cured hams could be detected by the quenching of fluorescence in tetraoctylammonium

fluorescein mercuric acetate (Sensors and Actuators B 38-39 (1997) 390-394). However, such a sensor compound would never be acceptable for use inside food packaging. Further, we believe that it would be more desirable for retailers to be able to detect spoilage by detecting the appearance of fluorescence or the appearance of a chromophore than by detecting the quenching of fluorescence.

Microbial growth on food and chemical degradation tends to result in the formation of volatile spoilage products. We have invented a product and method which utilises such spoilage products within the pack to sense food spoilage. Although the present invention will be described hereinafter with particular reference to food spoilage, it should be understood that its principles may be more widely applied. Thus it is contemplated that the invention may be applied to detecting the opening or the compromise of sterile packaging of instruments, dressings or drugs, in the microelectronics industry, as an aid to the quality assurance process in food factories, and in security packaging for papers, securities, banknotes, and other valuables.

The present invention provides a sensor for detecting food spoilage or the opening or compromise of packaging, comprising a metal co-ordinated complex immobilised in or on a substrate, which complex is capable of releasing a detectable component by the preferential binding of a gaseous substance to the metal of said complex. The complex may be, for example, a metal complexed with a chromophore or fluorophore, which undergoes ligand exchange with sulphur compounds (*eg* sulphides) or nitrogen compounds (*eg* amines), thus releasing the chromophore or fluorophore to indicate spoilage. Other gases relevant to the present invention contain alcohol or carbonyl groups or contain phosphorus.

Desirably, the complex is immobilised in the form of a film, which may be formed by printing, casting, roller application, brushing, spraying or like techniques, a composition comprising the complex onto the internal surface of the food package. In another embodiment, the complex is incorporated into, or into part of, a food packaging material itself. The invention therefore also provides such a composition for application onto food packaging, comprising the complex, an immobilising resin and a liquid vehicle. The system

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used for immobilising the complex may also retain and immobilise the chromophore or fluorophore. If required it is possible to incorporate some form of barrier layer or coating which is permeable to the food spoilage products but not to the indicator molecule or metal compounds.

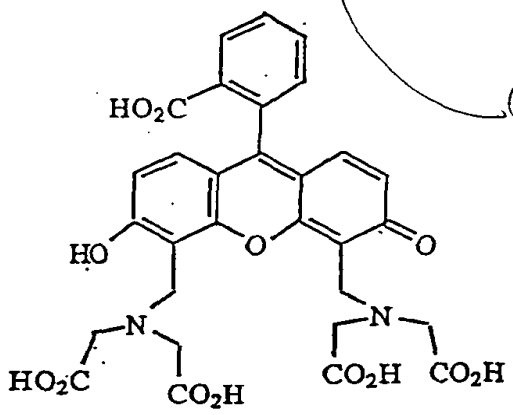
A variety of metals may be used to form the complex, and include especially palladium, platinum, ruthenium or iron, but other metals may be considered, such as copper, nickel, zinc, gold, the rare earth metals, cobalt, iridium, titanium and vanadium.

Some retailers may desire that the complex releases a fluorophore which does not show any appreciable colour change under normal shop lighting, but fluoresces strongly when excited by non-visible light such as UV. This permits the retailer to scan packages, *eg* by a portable UV lamp, and remove those that show release of the fluorophore caused by food spoilage products. For other areas of use, release of a chromophore, giving a visible colour change, may be more desirable. A variation on release of a fluorophore is the reaction of the complex to cause a shift in the position of an emission peak. This may be sufficient to be visible by eye when the fluorophore is excited, but the invention also encompasses the detection of such a shift by an instrument. It is to be understood that the term "chromophore" as used in the present invention includes compounds which exhibit phosphorescence.

The release of the chromophore or fluorophore is desirably not specific to any type or species of microorganism. The invention is believed to be sufficiently flexible to permit the development of a variety of sensors, either which indicate directly the level of microorganism growth or which switch "on" at a given level; for example a strip of sensors may indicate increasing levels of contamination up to a danger level.

Desirably, the complex also may be designed for particular uses, and to achieve particular results. For example, a particular palladium-fluorophore complex exhibits very much faster kinetics for fluorophore release than the corresponding platinum-fluorophore complex. According to the intended use and the preferred kinetics either, or both,

complexes may be used to yield particular preferred results. The complexing ligand is not itself critical providing it is released from the metal in the appropriate time-frame, and provides on reaction with spoilage products the desired fluorescence or colour change characteristics. A preferred ligand is Fluorexon, of general formula




This may be reacted with $Na_2[PdCl_4]$ to yield a Pd-Fluorexon complex which is pink in colour but which fluoresces strongly when the ligand is released. The Fluorexon molecule can itself be modified so that it is no longer water soluble, but is soluble in lipids or organic solvents, for example by using a non-co-ordinating counterion or by changes in functional groups, as is well known to the skilled chemist. The preparation of such a palladium complex is described in more detail in the following Example.

Other palladium complexes may be considered for use in the present invention are known from the literature, for example palladium dializarin red, $(NBu_4)_2[PdAlizarin_2]$ and the palladium complex of alizarin complexone. Generally, the complex may be any suitable complex of a dye, a complexone, a Schiff base, or could be a rare earth polyamino carboxylate. Particular complexing fluorophores to be considered in addition to Fluorexon are know *per se*, and include a number of compounds commercially available, such as fluorescein isothiocyanate, fluorescein, fluoresceinamine, calcein blue, "Fura 2", quinzarin, alizarin complexone, alizarin red and alizarin, isocein, "Quin 2" and 4,4-dihydroxy-azobenzene 3,3-dicarboxylic acid, disodium salt.

The presently preferred Pd-Fluorexon complex may be dissolved in an aqueous PVA solution, to form a composition which can be applied to plastics packaging materials to yield a water-insoluble film. It is envisaged that other such compositions, with other metal complexes, may be established by trial and error, and it is convenient to use generally available ink-forming technology. Such an ink may be applied to the inner surface of a package, or printed or otherwise applied onto a label for insertion into a package. Such inks or compositions may contain other components, including particularly one or more of driers, plasticisers, fillers, surfactants and pigments. In addition to labels to be packaged inside packaging, the invention includes adhesive labels, decals and the like.

Alternatively, incorporation of the complex into the packaging material may be considered, providing that when so incorporated, there is sufficient permeability to cause the complex to release the desired detectable component.

The present invention will now be described by way of example only.

## EXAMPLE 1

### A. Preparation of Solution of Pd:Fluorexon

4'5'-Bis(N,N-bis(caboxymethyl)aminomethyl fluorescein (0.1g, 1.6 x $10m^{-4}$) and $Na_2(PdCl_4)$ (0.12g, 3.2 x $10^{-4}$m) were suspended in $H_2O$ ($90cm^3$) and heated under reflux for 30 minutes. The suspension was filtered whilst warm, resulting in a red/pink solution. A tarry dark red/brown residue was removed during filtration. The resulting solution is approximately 1.6 m M.

### B. Preparation of Solution of Pd:Fluorexon in PVA

4g of the solution prepared in A above was added to a commercial 6% PVA (16g, Rhone Poulenc 25-140 Rhodoviol) in $H_2O$ solution, and mixed in a high shear mixer for 5 minutes.

**C. Production of Film**

0.5cm$^3$ of the mixture resulting from B above was drawn into a film on a polyester film sheet (Mylar) using a K-bar size 3, and left to dry at room temperature. A smooth film coating was formed, pale pink in colour.

**D. Tests for Spoilage Products from Meat**

A variety of tests were carried out on samples of fresh minced beef and chicken purchased from a local butcher. The samples were sub-divided and left with the existing natural flora. The samples were either refrigerated at 4°C or stored at room temperature in closed vessels in which was located a 1cm x 1cm label cut from the film produced as in C above.

**E. Fluorescence Testing**

E(i) Initial tests were carried out on a Fluorexon solution in water (a) and the Fluorexon solution immobilised in a film produced from 10% PVA in analogous manner to C above (b), and fluorescence peaks were determined. These are plotted in accompanying Figure 1. It can be seen that there is a distinct fluorescence peak at about 520nm for the solution and at about 530nm for the film, demonstrating a slight shift because of the matrix of the film.

E(ii) Samples of the Pd:F solution prepared in A above were taken. One was retained as a control (a) and other samples were admixed with $10^{-6}$ M diethylamine. Fluorescence was measured at various times and the fluorescence spectra are plotted on accompanying Figure 2. It was readily seen that there is an increasing intensity with time, demonstrating the release of fluorescent ligand from the complex. Similar results have been obtained when the diethylamine was replaced with the amino-acid cysteine.

E(iii) The fluorescence of the labels used in the tests described in D above was established. In the case of the meat stored in the refrigerator, the fluorescence plots are

shown in Figures 3 and 4 at 24 hours and at 168 hours (seven days) respectively. It is to be noted that in Figure 4 the Y scale is very much expanded in comparison to Figure 3. A very small peak in shown for the film exposed to chicken breast (a) in Figure 3, but there is no significant fluorescence from the film exposed to minced beef (b). A control of film sample stored over sterile water (c) is shown for comparison. However, by 168 hours, there has been a dramatic increase in intensity in fluorescence in both cases. Both sample looked and smelled "spoilt" by this stage.

In the case of the meat stored at room temperature for 24 hours, the label fluorescence plots are shown in Figure 5. Both chicken breast (a) and minced beef (b) show dramatic peaks at about 550nm. The control (c) of a label over sterile water does not show any corresponding peak. Although the intensity of the fluorescence from these meat labels is not so great as that resulting from seven days in the refrigerator, it is clear that the spoilage process has begun and that the Pd complex is being affected by spoilage products to release the fluorophore.

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